REGENERATION IN THE HYDROMEDUSA, GONIONEMUS VERTENS.

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HAECKEL,[1] in 1870, stated that he found the power of regeneration remarkably developed in several species of medusæ belonging to the family Thaumantidæ. He discovered that if a medusa be cut up into more than a hundred pieces, each piece, provided it contains a part of the margin of the bell, will develop a complete medusa ("*eine vollständige kleine Medusa*"). The little medusa developed in a few (two to four) days. Even a single tentacle, if it contained at its base a small part of the margin of the bell, would make a new medusa. No details are given, and it is not possible to gather from the account whether new organs developed, as one would expect, if the little medusa was complete (*vollständig*), or whether only the medusa-form was assumed by the pieces.

Haeckel also added that if the segmented egg, or even the ciliated larva, was cut up into many pieces, each piece would make a new small larva.

Hargitt[2] described in 1897 the results of a number of experiments that he had made on the regeneration of the medusa Gonionemus. He found that excised portions of the margin of the bell regenerated promptly, but it is not clear in this case whether he meant by regeneration that the cut edges closed together, or whether the parts cut off were replaced. When the medusa was cut into two equal pieces, each became an "independent and perfect medusa." The restoration was somewhat peculiar. "It would seem to be a *recovery* of form and function rather than regeneration in the usual sense of that term." "The new medusæ were in most respects quite simi-

[1] Monographie der Moneren., *Biologische Studien*, Heft 1 (1870), p. 23.

[2] Hargitt, C. W. Recent Experiments on Regeneration, *Zoölogical Bulletin*, 1897, vol. i.

lar in form and action to the original, though of course only about half the size. The time at my disposal was insufficient to observe whether there was subsequent growth of the specimens. In the recovery of the specimens I was not able, moreover, to observe any disposition to regenerate the additional radial canals necessary to complete the symmetry of the original. This, however, does not seem to be an important matter, since there does not seem to be a special necessity for a definite number."

Hargitt also cut the medusa in two in a horizontal plane — one piece being bell-shaped and the other a ring. The former showed evidence in one case of forming new tentacles; the latter produced a new medusa. Referring to the latter, Hargitt states that "the process appeared as more a restoration of form" than the formation of a typical medusa. Neither mouth nor gastric cavity developed. The figure given to illustrate this shows a small medusa with only fourteen tentacles around the margin, while the original piece contained thirty-eight tentacles. The absence of twenty-four tentacles is not accounted for, and is a point of some theoretical interest, since one of the important problems in connection with the development of a small medusa out of the ring is whether the old organs are retained intact or changed over into new ones proportionate in number and size to the smaller dimensions of the new individual. Hargitt also showed that if the manubrium is excised close to the stomach it is regenerated (*i.e.*, a new one develops).

These interesting experiments of Hargitt, although lacking in some details, show clearly that pieces of the medusa as small as one-fourth the whole have a remarkable recuperative power, leading to the production of the bell-like form. The account leaves the question open, whether these bell-like individuals will produce the missing organs if kept for a longer time. My object in studying the process of regeneration in this jellyfish was to find out more definitely by what means it regained its medusa form; whether by the development of new tissues and new organs, or whether by a rearrangement of the old part. Further, to find out if, after some time, the organs of a typical

medusa reappeared, and to examine the behavior of pieces taken from different parts of the body. It was not clear to me exactly what took place, or how the change was brought about; and the results show that the problem is not a very simple one.

Gonionemus has generally four radial canals diverging from a well-defined central stomach. From the latter hangs down a

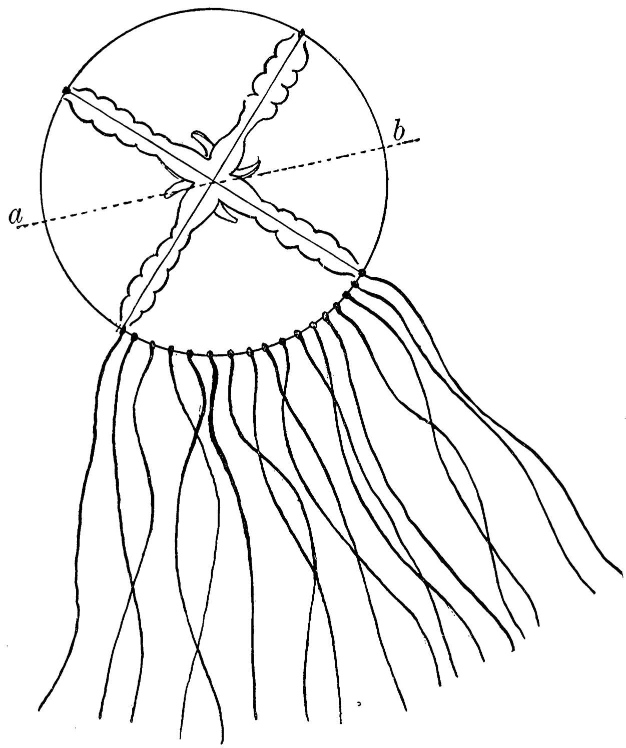

Fig. 1.

short manubrium. The individuals vary in size. I have generally used those of medium and large size (10 mm. to 20 mm. in diameter). The number of tentacles also increases as the medusa grows larger. In a very small individual there were twenty-four tentacles; in one a little larger, thirty-two tentacles; in a larger one, forty tentacles; and in quite a large specimen there were about sixty-four tentacles.

When a medusa is cut in two in a vertical inter-radial plane,

as indicated by the line *a–b* in Fig. 1, the cut surfaces bend slowly inwards and towards each other, and in the course of twelve to twenty-four hours they have met and fused along their entire length. As a result, the semicircle of tentacles now forms a complete smaller circle. The cut edges of the velum also meet, leaving an opening in the center of the velum, as in the typical medusa. The general form of the new individual is like that of the typical form, except on one side the bell is at first less rounded. It can swim about, eat, and I have been able to keep them alive for several weeks. A glance will show, however, that a typical medusa like the one from which the piece was taken has not been formed, for only two radial canals are present that extend out from the stomach (Fig. 2). The latter does not lie at the top of the subumbrella space, but somewhat to one side (Fig. 3). From the stomach arises a new manubrium. If the old manubrium was cut in half when the medusa was divided, each half makes a complete manubrium ; but if, as is often the case, the cut passed to one side of the original manubrium, then one piece retained the old manubrium, and the other piece developed an entirely new one.

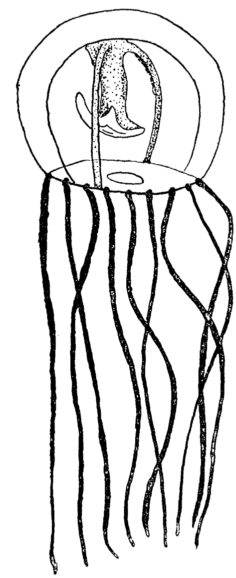

FIG. 2.

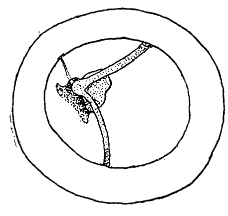

FIG. 3.

Along the line where the cut edges fused together a scar is present that resembles somewhat a third radial canal, but the third canal did not develop, although in some cases a short diverticulum may extend from the stomach for a short distance along this line. If the tentacles be counted after the operation, and then again when the piece has healed, the number will be found to be the same, and this holds true for several weeks. It is possible of course that a few new tentacles may develop, since they develop also in the normal individual as it

grows larger. Occasionally one or two new tentacles are found in the region where the parts have united, but as a rule I have not noticed any increase in the number.

If a medusa be cut into four parts, each containing one of the radial canals, then each piece gives rise to a small medusa-like individual. The cut edges come together and fuse ; the tentacles form a circle, and a velum is also formed with an opening in the middle (Figs. 4 and 5). The new manubrium arises from one side of the bell (Fig. 4), and not from the top of the subumbrella space. Its point of origin is determined by the position in the new individual of the portion of the original stomach. During the process of fusion of the cut edges the proximal end of the radial canal is carried in some cases far over to one side of the new bell. In such a case the manubrium appears to arise just inside of the line of tentacles. In other cases the proximal end of the radial canal is not carried so far, and in such cases the manubrium hangs down from higher up in the subumbrella space. While the form of the one-fourth medusa has in general the typical bell-shape, yet such individuals have only one radial canal and an eccentric manubrium. It may be thought that the process up to this time is only one of healing, and that later the missing parts would regenerate. In the hope of seeing if this were true, I kept alive some of these medusæ for several weeks, and although they seemed to be in excellent condition, yet they did not show the least sign of regenerating the organs that make a complete medusa.

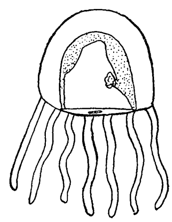

FIG. 4.

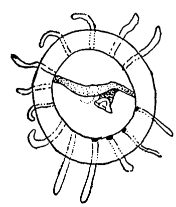

FIG. 5.

These experiments suffice to show that while the healing power of the one-half and one-fourth fragments is very great, yet the regenerative power is not well developed, for neither do the old parts change over into new ones having the typical arrangement (except in so far as the medusa-form is produced), nor do the missing parts regenerate (except the regeneration of a new manubrium) where the edges have healed together.

If only one quadrant is cut out, the larger part (three-fourths piece) forms a medusa having three radial canals and three-fourths the number of tentacles.

Other experiments were made to see if smaller pieces than one-fourth would develop into the form of medusæ. These smaller pieces were cut off in different ways and from different parts of the medusa. If the jellyfish is taken from the water and laid on its side on a glass plate, a portion of one side may be easily cut off in the way indicated by the line *a–b* in Fig. 6. A piece cut off in this way will contain only a part of one radial canal, but somewhat more than one-fourth of the margin with its tentacles (approximately one-third in this case). If the plane of division is less oblique, as indicated by the line *c–d* in Fig. 6, then the entire piece in all its parts is smaller than one-fourth the entire medusa. In both experiments a small bell-shaped individual develops from the piece. The new manubrium regenerates from the cut end of the radial canal, and lies to one side of the new medusa. This shows that a new manubrium may develop from the radial canal some distance from the original stomach.

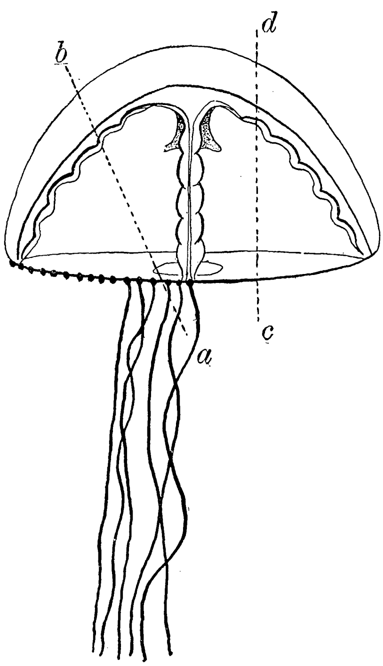

FIG. 6.

Small pieces were also removed in another way. The proximal part of the bell was cut off from the distal part, as shown by the line *a–b* in Fig. 7. Then the distal ring was cut up into smaller pieces, as indicated by the vertical lines in the same figure. In one case the ring was cut into four equal parts, each with a part of a radial canal. The pieces closed in, but somewhat imperfectly, and although they were kept for twenty

days they did not assume the typical medusa-form. In another case the distal ring was cut into eleven pieces. Four of the pieces contained the distal end of a radial canal; the rest did not contain any part of the radial canals. About half of the pieces showed later little resemblance to the medusa-form. They became swollen and irregular, and although kept for twenty days did not develop further. Other pieces became somewhat bell-shaped, but in none of them was the medusa-form well developed. These pieces seemed, therefore, to be near the lower limit of size necessary for the formation of even the medusa-form, although it is possible that even smaller pieces of a different shape might produce the characteristic form.

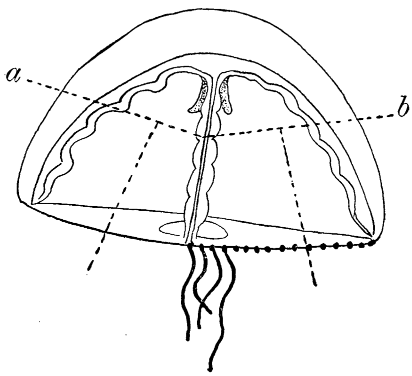

FIG. 7.

The following experiment was made to see if, in the absence of a definite structure such as the radial canal, a piece might regenerate a new radial system having the typical form. A jellyfish was placed oral side down on a glass plate, and with a knife, or with scissors, an inter-radial piece triangular in outline was cut out (Fig. 8, *A*). A piece of this sort does not contain any part of the central stomach or of the radial canals. It contains a part of the endoderm in the form of a plate extending throughout the middle of the piece, and a ring canal around the margin. In all, forty-eight pieces of this kind were cut out. Most[1]

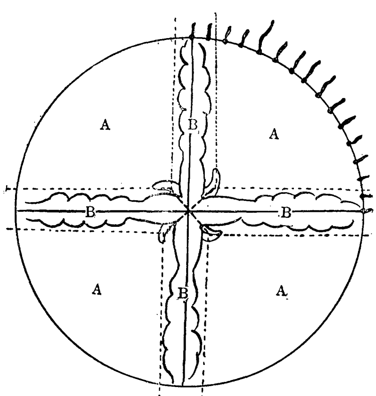

FIG. 8.

[1] Some of them died, especially in one experiment, due to too many being kept in the same dish.

of the pieces closed in and the old tentacles formed a ring encircling the margin. The piece assumed, in a general way, the form of the medusa (Fig. 9), and from the middle of the lower side a proboscis-like outgrowth developed (stippled in the figure). In the center of the bell two cavities are present. In order to interpret these structures it was necessary to make serial sections. The sections show that the larger cavity (stippled in the figure) represents the subumbrella space, and is therefore lined by ectoderm. This ectoderm continues down into the proboscis-like structure of the lower side.

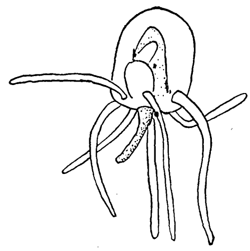

FIG. 9.

The outer surface of this structure is covered by the outer ectoderm. It represents, therefore, not a new manubrium, but a tubular outgrowth of the velum, the latter having completely closed over the lower surface. The outgrowth is fringed along one side and might easily be taken for a new proboscis, but sections show, in the clearest way, that it does not represent that organ. The other cavity seen in the figure lies on the side where the piece closed in. It represents an enlargement of the ring canal and is continuous with the ring canal around the base of the tentacles. The results show that the small piece, while assuming the form of a bell, is completely lacking in the essential organs of the medusa.

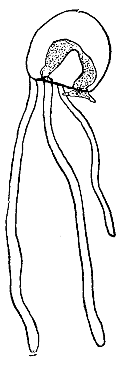

FIG. 10.

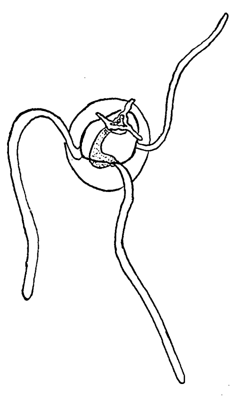

FIG. 11.

In this same experiment the cross-shaped piece that remained after the four triangular pieces had been removed was itself cut into four pieces through the stomach (Fig. 8, *B*). These small pieces sometimes developed into medusa-like forms, each with a manubrium, but did not reproduce the other missing parts (Figs. 10 and 11).

Quite a number of experiments were made at different times

to see if the proximal end of the bell, with its contained organs, *viz.*, the stomach, manubrium, and the proximal ends of the radial canals, could develop a new circle of tentacles, sense-organs, etc. Hargitt has described one case in which there was some evidence of the development of new tentacles, but the piece died without further development.

In this experiment I cut off the proximal bell portion, sometimes by cutting around with a pair of scissors, sometimes by laying the jellyfish on its side and cutting off the margin with a knife.

Some of the proximal pieces were small, some large, some had been cut off by a nearly circular line, others by two or three oblique lines. In most cases the bell portion contracted, after a day or two, around the base of the manubrium, but no further development took place. In other cases the cut edges met and fused to form a sphere entirely closed. These kinds of pieces disintegrated after several days. Forty pieces in all behaved in one or the other way. It will be noticed that in nearly all these cases the part removed had been cut off less than halfway down the side of the bell, or near that level. It occurred to me that if the margin of the bell were cut off not so high up as before, but farther down, better results might be obtained, both because larger proximal pieces would result, and also, the cut edge being nearer to the old tentacles, the new ones were more likely to develop. In thirteen individuals the margin was removed as just described. Two of these showed, five days after the operation, small opaque projections around the margin of the cut edge. In one of these (Fig. 12) there were twenty of these small knob-like bodies. Although these grew somewhat more distinct and slightly larger during the next day or two, they failed to develop further. These two cases are similar to the one described by Hargitt. He attributed the failure of the knob-like projections to develop into tentacles to the unprecedented hot weather that prevailed at the time. But although I kept one of the two pieces for

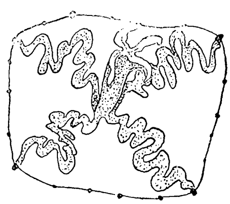

FIG. 12.

a week or more after the knobs appeared, they failed to develop. Considering the large number of experiments that I have made, I am inclined to think that Hargitt overestimated the capabilities of pieces of this sort. The result, it seems to me, follows only when the cut has been made near the margin of the medusa. The peripheral rings that had been cut off in the preceding experiments, fifty-three in number, were kept alive to see if, as stated by Hargitt, they too would give rise to medusæ. All those pieces from which a large part of the bell had been removed failed entirely to close in, and died after a few days. It seems that under very favorable circumstances a piece from which only a small proximal part has been removed may again develop into a complete medusa, and in fact, in one or two cases, this seemed very nearly accomplished. In one case a new stomach formed, and a very small manubrium; in another case the proximal ends of two of the canals united, and a small manubrium developed, but lay somewhat eccentric in position. The difficulty seems to be in the closing in of the ring rather than in the regeneration of a new stomach and manubrium. This is shown by the following experiment:

In several cases I cut out from the oral side the entire stomach and its attached manubrium, as well as the immediate proximal ends of the radial canals. Care was taken to remove completely all the endoderm in this region, but the cut did not pass through the jelly of the bell. I hoped to see if, under these circumstances, a new stomach and manubrium would develop, or if from each of the proximal ends of the radial canals a single manubrium would sprout forth. The latter possibility would seem to exist in the light of the experiments on the one-half and one-fourth pieces. If, on the other hand, a new stomach and a single manubrium developed, this would seem to indicate some sort of interrelation of the parts with one other (the canals are, of course, connected by a plate of endoderm). The endoderm grew forward over the region previously occupied by the stomach, and out of it was formed a stomach from which a new manubrium grew out.

It has been shown that in the one-half and one-fourth

medusæ the new manubrium develops always from the cut end of the radial canal. It often lay far to one side of the center of the new bell. I tried in another experiment, cutting in two the radial canal in the one-fourth piece at the middle of the canal, *i.e.*, at a point that would correspond approximately to the middle of the new bell. Under these conditions it was possible that a new manubrium might develop at this point, rather than at the other proximal end of the tube. This result did not follow, however, since, at the cut, the ends came together and fused. The experiment might succeed if the cut ends could be kept apart, or if a short piece were cut out of the canal at some point.

Finally I cut off the rim with its tentacles from three of the four quadrants, leaving only one quadrant entire. The experiment was made to see if, when a part of the rim was left, it might give rise at its free ends to the missing part of the ring, or possibly, under these conditions, the injured part of the rim might more readily produce new tentacles. The medusa closed in so that the opening into the subumbrella cavity became quite small. The opening was surrounded for the greater part of its extent by the tentacles of the uninjured quadrant. Over a small region no tentacles were present at first, but after five days a few new ones appeared. They were not more numerous near the ends of the uninjured part of the ring than elsewhere. The result is similar to that in which the entire margin is cut off (Fig. 12), although in this case the new tentacles were better developed.

The results of these experiments show, for Gonionemus at least, that Haeckel's statement, that even the smallest pieces may make new medusæ, is not correct ; for although pieces somewhat smaller than one-eighth of the medusa may make new individuals having the medusa-form, yet these small individuals, as well as larger ones, lack the most essential features of the medusa. The remodeling extends only to the form of the entire piece and does not include the internal organs.

It is puzzling to determine whether the medusa-form assumed by the pieces is simply the form that necessarily results after the fusion of the cut edges, or whether the process includes

something more than this. When I recall how similar in form the small medusæ are, coming as they may from pieces of very different shapes, I am inclined to believe that there is something more in the process than only the fusion of the cut edges, and that the piece does in reality mould itself into the medusa-form, as Hargitt pointed out.

The further question suggests itself for consideration: Is the process by which the edges bend in and ultimately fuse simply a mechanical result depending upon the tensions set in play when part is cut off? I think not, at least not entirely. The bending takes place very slowly, and not quickly, as would be the case were it simply a roughly mechanical process. The form of the piece continues to change even after the edges have met and fused, pointing, I think, to the conclusion that the entire process is one of rounding up of the piece in the direction of least resistance.

The meeting of the edges may sometimes be due simply to an accidental meeting of the bent-in portions, but generally the process is a more orderly one, and of such a sort that it seems to be correlated with the process of remoulding. The fact that the cut edges always succeed in finding each other, even in very unsymmetrical pieces, shows that something more than a gross physical process is at work.

If we attempt to analyze the process so far as is possible at present, we can, I think, make out the following factors. The bending in, taking place in the piece as a whole, seems to be the result of active changes in the living tissues. This leads to the piece assuming more and more the typical form. The meeting of the edges may be due sometimes to accidental contact resulting from the bending in of the piece, but more often the closing of the cut surface is due to a sequence of well-defined events. At some point where two cut edges make an angle with each other they begin to draw together at the angle. The process proceeds from this point until, step by step, the complete fusion is brought about. It is as though, first at the angle, the parts that are continuous around the angle draw together, and the edges coming together fuse. Then new parts that are brought up to the new angle repeat

the process until the entire cut edges are drawn together. There is no evidence that parts widely separated attract each other across the intervening region, as in cases of cytoplasm described by Roux. The cytoplasm is confined to those parts in contact, or at any rate so near together that they may be connected by protoplasmic processes.

I have said enough to show that the process by which the piece closes in is a complex one, in which several factors take part. It is towards the discovery of these simpler processes that take place during regeneration that we can at present, I think, most profitably direct our attention. For if, as appears to be the case, many components enter into the process that we call regeneration, we can only hope to understand the phenomena as a whole when we have resolved it into the immediate simpler factors of which it is made up. Then no doubt we shall find out that we can in turn resolve these factors into simpler ones, and our analysis will be carried a step farther. As long as we interest ourselves with the facts and factors of regeneration the work is not likely to come to a standstill, but when we leave the analytical method and attempt to construct injudicious theories that make the pretense of explaining a complicated process without attempting to resolve the process itself into its factors, then progress stops. Such, I believe, to be the case in the attempt to explain the process of regeneration by a theory of preformed imaginary germs. A theory of this kind is only a pretense; imagination takes the place of verifiable hypothesis, and the process that we set out to study is explained by saying that there are "germs" present that have been set aside to bring about the result!